 Bull. Chem. Soc. Jpn., 75, 59–63 (2002)

Dynamics of the Hydration of Halogenoalcohols in Aqueous Solution

Shoichi Okouchi,* Tatsuya Ashida, Shinobu Sakaguchi, Kazushi Tsuchida, Yoshimasa Ishihara, and Hisashi Uedaira

Department of Materials Chemistry, Faculty of Engineering, Hosei University, Kajino,Koganei-shi, Tokyo 184-8584

(Received June 11, 2001)

The ^{17}O NMR spin-lattice relaxation times (T_1), of the solvent water in aqueous solutions of halogenoalcohols were determined as a function of the concentration at 298 K. The values of the dynamic hydration number, $n_{DHN} \equiv n_h(\tau_c^h/\tau_c^0 - 1)$, were determined from the concentration dependence of T_1. In order to investigate the effect of the halogen atom on hydrophobic hydration, the ratios (τ_c^h/τ_c^0), of the rotational correlation times,(τ_c^h), of water molecules around the solute to those of pure water,(τ_c^0), were estimated from the n_{DHN} and the coordination numbers (n_h), which were calculated from the water-accessible surface area (ASA) of the solute molecule. (i) The τ_c^h/τ_c^0 value decreased in the order EtOH > IEtOH ≥ BrEtOH ≥ ClEtOH > FEtOH for monohalogenoethanols, EtOH ≥ Cl_3EtOH > Cl_2EtOH ≥ ClEtOH and EtOH ≥ F_3EtOH > FEtOH for polyhalogenoethanols, and PrOH > ClPrOH and *i*-PrOH > $(CF_3)_2$CHOH for halogenopropanols. (ii) The ASA dependence of the τ_c^h/τ_c^0 value for chloroethanols was smaller than that for fluoroethanols. These results are explained by the disturbance of hydrophobic hydration by the halogen atom and the size effect.

The various effects, such as denaturation, the helical content and the enzyme activity of the proteins, result in aqueous solutions of halogenoalcohols and halogenoacetic acids.[1–3] Aqueous solutions of halogenoalcohols and halogenoacetic acids change the helical content and the enzyme activity of the proteins.[1,2] Anesthetics, such as chloroform and fluothane, transform α-helix polylysine into a β-sheet,[3] and have a variety of biological activity. Although the hydration of these compounds, particularly their hydrophobicity, is thought to play an important role in the interaction with biopolymers, the detailed mechanism is still unclear.[4]

There are various opinions about the hydrophobicity or the structure making effect of halogenoorganic compounds. Wen and Mucaitelli[5] showed that the hydrophobicity of perfluorocarbons is greater than that of the corresponding alkans from the temperature dependence of the solubilities. The strength of the hydrophobic interaction between the fluorocarbon surfaces is slightly different from that between hydrocarbon surfaces.[6] Muller[7] determined the octanol-water partition coefficients of trifluoromethylated aliphatic alcohols. He showed that trifluorination enhances the hydrophobicity and that the degree depends on the position of the trifluoromethyl group in the molecule. Mizuno et al.[8,9] found that the chemical shift of the ^1H and ^{17}O nuclei of water in aqueous halogenoalcohol solutions shifts to high-field with increasing the concentration, contrary to those of the alcohols, and concluded that the halogenoalcohols break the water structure. Thus far, although many studies on the hydration of fluoroalcohols have been carried out,[7,10–12] only a few studies on other halogenoalcohls have been conducted.[8,9] It is important to elucidate how the hydration of halogenoalcohls changes with the kind and number of halogen atoms.

Recently, we measured the spin-lattice relaxation time, (T_1), of ^{17}O nuclei of water in aqueous solutions of amines, diamines,[13] alcohols and diols,[14] and obtained the dynamic hydration numbers, (n_{DHN}), and the ratios(τ_c^h/τ_c^0), of the rotational correlation times,(τ_c^h), of water molecules around the solute to those of pure water,(τ_c^0). We showed that the dynamic state of water molecules around the hydrophobic group depends on their size and shape, and is disturbed by the neighboring hydrophilic group. Since the τ_c^h/τ_c^0 values for halogenoalcohols have not been obtained, it is necessary to investigate the dynamic state of water molecules around the halogenoalcohols in accordance with the course of these investigations.[13–15]

In the present paper, we report on the results of T_1 measurements of the natural abundance ^{17}O nuclei in aqueous solution of halogenoalcohols at 298 K. We obtained the n_{DHN} of these compounds. We also calculated the water-accessible surface areas (ASA) and the coordination number, (n_h), of these compounds, and obtained the ratio of the rotational correlation time(τ_c^h/τ_c^0), from n_{DHN}. We discuss the effect of the kind and number of halogen atoms and the molecular size on the hydration of halogenoalcohol. In addition, we discuss the relation between the alcohol-induced denaturation of proteins and the hydration of halogenoalcohols.

Method

The T_1 of ^{17}O nuclei of water molecules in aqueous solution is adequately represented by an empirical equation of the form

$$T_1^0/T_1 = 1 + n_{DHN} \cdot x/(1-x). \quad (1)$$

Under extreme motional narrowing conditions, we obtained the following relation according to the two-state model:

$$n_{DHN} = n_h(K \cdot \tau_c^h/\tau_c^0 - 1), \quad (2)$$

where x is the mole fraction of solute, K is the ratio of ^{17}O-

Table 1. Hydration Characteristics of Halogenoalcohols in Aqueous Solution at 298 K

No.	Compounds	ASA (Å^2)	n_h	n_{DHN}	τ_c^h/τ_c^0
1	CH_3CH_2OH*	205.8	15	10.3	1.69
2	$HOCH_2CH_2OH$*	218.7	16	5.6	1.35
3	FCH_2CH_2OH	210.8	15	5.6	1.37
4	F_3CCH_2OH	219.3	16	10.2	1.64
5	$ClCH_2CH_2OH$	232.5	17	8.9	1.53
6	Cl2CHCH$_2$OH	254.4	18	10.5	1.57
7	Cl_3CCH_2OH	272.5	20	13.3	1.68
8	$BrCH_2CH_2OH$	242.8	18	9.5	1.54
9	ICH_2CH_2OH	254.3	18	10.2	1.56
10	$CH_3CH_2CH_2OH$*	237.0	17	14.1	1.82
11	$HOCH_2CH_2CH_2OH$*	250.5	18	10.2	1.56
12	$ClCH_2CH_2CH_2OH$	264.0	19	11.1	1.58
13	$(CH_3)_2CHOH$*	233.1	17	15.8	1.94
14	$(CF_3)_2CHOH$	253.7	18	13.7	1.75

*Ref. 9.

quadrupole coupling constant ^{17}(O QCC) of the hydration water to that of the bulk water, and superscripts h and 0 refer to the hydration water and bulk water, respectively. Bagno et al.[16] calculated ^{17}O QCC for methanol–water and formamide–water systems using ab initio calculations. From the results, they showed $K = 1$ in aqueous solutions of alcohols, amides etc. We assume $K = 1$, since aqueous halogenoaclohol solutions are dilute.[13–15]

The n_{DHN} is the dynamic hydration number which has the following physical meaning.[15,17] Consider a hypothetical molecule of the same size and shape as the solute molecules, which interacts with water in the same manner as would a water-water interaction. Then, n_{DHN} represents the differences in the dynamic states of the water molecules in the hydration layers of the solute and of the hypothetical molecule. It corresponds thermodynamically to the entropy difference of water molecules in both hydration layers.

The coordination numbers, (n_h), is determined from the accessible surface areas, (ASA), because it is very difficult to obtain the values of n_h experimentally.[13,14] A computer program QCPE No. QCMP 132 was used for the ASA calculation with a water-molecule radius of 0.15 nm. The calculated values of the ASA and n_h for halogenoalcohols are given in the 3rd and 4th columns of Table 1.

Experimental

Halogenoethanols and halogenopropanols were purchased from Wako. All compounds were of GR grade and were used without further purification. All aqueous solutions were prepared in deionized and ultrafiltered water.

All natural-abundance ^{17}O NMR experiments were performed using a JNM EX-270 spectrometer operating at 36.63 MHz. The T_1 of nuclei was measured with an error of ±3% at 298 K by using the inversion recovery sequence. The temperature was maintained at ±0.5 K by means of gas-thermostatting.

Results and Discussion

The values of T_1^0/T_1 for $H_2{}^{17}O$ in aqueous solutions as a function of the solute concentration at 298 K are shown in Figs. 1 and 2 for halogenoethanols and Fig. 3 for halogenopropanols. The straight lines in the three figures were calculated by the least-squares method. The slopes of those straight lines equal the values of n_{DHN} from Eq. 1, and the values of τ_c^h/τ_c^0 can be calculated using Eq. 2 from those of n_{DHN} and n_h.

The values of n_{DHN} and τ_c^h/τ_c^0 for halogenoalcohols are pre-

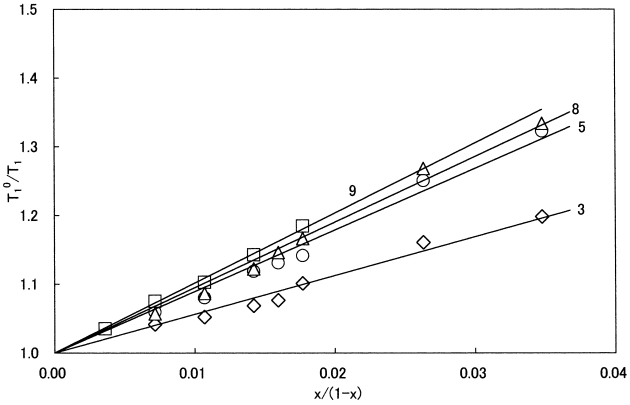

Fig. 1. T_1^0/T_1 for $H_2{}^{17}O$ in aqueous solution of monohalogenoethanols as a function of the solute concentration at 298 K. The numbers demote the same compounds in Table 1.

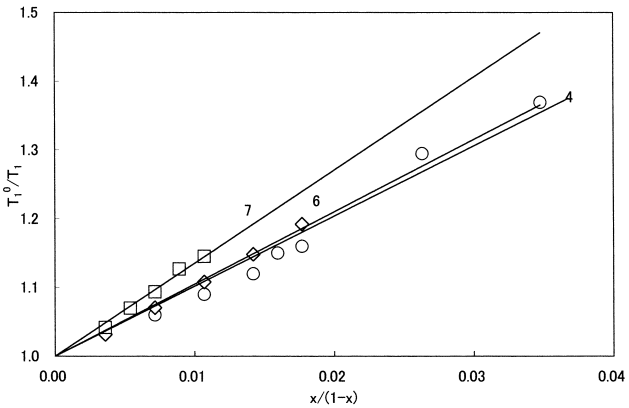

Fig. 2. T_1^0/T_1 for $H_2{}^{17}O$ in aqueous solution of polyhalogenoethanols as a function of the solute concentration at 298 K. The numbers demote the same compounds in Table 1.

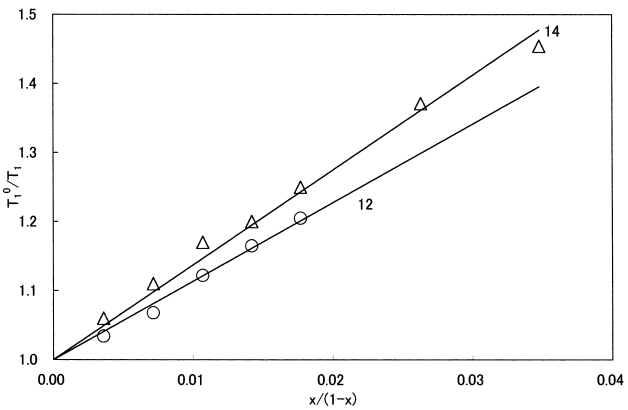

Fig. 3. T_1^0/T_1 for $H_2{}^{17}O$ in aqueous solution of halogenopropanols as a function of the solute concentration at 298 K. The numbers demote the same compounds in Table 1.

sented in the 5th and 6th columns of Table 1. For a comparison, the corresponding value for ethanol, 1,2-ethanediol, 1-propanol, 1,3-propanediol and 2-propanol are included in Table 1.[14]

As can be seen in Table 1, for all of the halogenoalcohols $n_{DHN} > 0$ and ${\tau_c}^h/{\tau_c}^0 > 1$. These results mean that the thermal motion of water molecules around the halogenoalcohol molecules is more inhibited compared with that in pure water, and the degree of inhibition depends on the kind and number of halogen atoms. We classify the results according to the kind and number of halogen atoms and discuss the effect of halogen atoms on hydrophobic hydration.

Dynamics of Hydration of Monohalogenoalcohols. The values of ${\tau_c}^h/{\tau_c}^0$ for monohalogenoethanols decrease in the following order, as shown in Table 1:

$$\mathrm{EtOH} > \mathrm{IEtOH} \geq \mathrm{BrEtOH} \geq \mathrm{ClEtOH} > \mathrm{FEtOH} > \mathrm{HOEtOH}. \quad (3)$$

The effect of the halogen atom on hydrophobic hydration becomes large in the order I < Br < Cl < F, which corresponds to that of the electronegatvity of the halogen atom.

A similar tendency to the relation of Eq. 3 holds for ClPrOH, namely,

$$n\text{-PrOH} > \mathrm{ClPrOH} > \mathrm{HOPrOH}. \quad (4)$$

These results are explained as follows. The arrangement of water molecules in the hydration layer of a hydrophobic group is characterized by local cage-like hydrogen-bonding structures.[18] The thermal motion of water molecules in the hydration layer is inhibited by the water–water interaction.

The hydrophilic group directly interacts with water molecules nearby. Thus, the structured layer of hydrophobic hydration is disturbed by an adjacent hydrophilic group, since this group can form hydrogen bonds with water molecules. A disturbing effect of the hydrophilic group results in a decrease in the ${\tau_c}^h/{\tau_c}^0$ value.[19–20] Owing to this effect, the ${\tau_c}^h/{\tau_c}^0$ values of diols are smaller than those of *n*-alcohols (see Table 1 and Fig. 5).[14] We showed that the hydration behavior of the diamines and the alkane disulfonate ions can be explained by this effect.[13,21]

Since the halogen atoms are the strong electronegative, they directly interact with water molecules nearby. The hydrophobic hydration is therefore disturbed by the halogen atom close to the alkyl group. The degree of disturbance by the halogen atom depends on its electronegativity and the effect of most electronegative fluorine atom is the strongest. As a result, the thermal motion of water molecules around the halogenoethanol molecule becomes greater and the ${\tau_c}^h/{\tau_c}^0$ value becomes smaller than that of ethanol.

Although the ${\tau_c}^h/{\tau_c}^0$ value of fluoroethanol is the smallest among those of halogenoethanol, the effect of a fluorine atom in a halogenoethanol molecule on the relaxation time of water molecules is weaker than that of a hydroxyl group (see Table 1). The disturbing effect is diagramed in Fig. 4, where the dotted lines represent the range disturbed by the halogen atom and the hydroxyl group.

Mizuno et al.[9] measured the chemical shift of water protons in the aqueous chloroethanol solutions and showed that the effect of the OH group of chloroethanol on the water structure is almost the same as that of the OH group of ethanol. This result is thought to be applicable for BrEtOH and IEtOH, since the electronegativity of the Br and I atoms is smaller than the Cl atom.

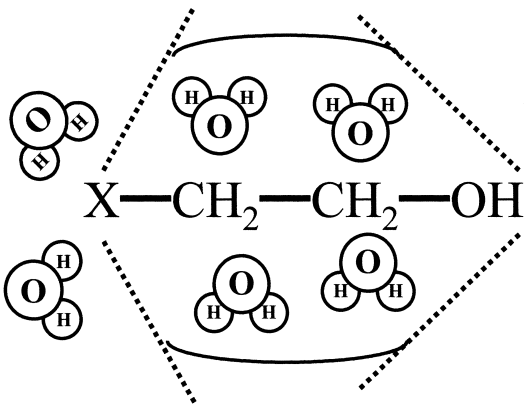

Fig. 4. Diagrammatic representation of hydrophobic hydration of halogenoalcohol. Dotted lines: the range of the disturbance by the halogen atom, X, and the hydroxy group.

The OH group of FEtOH may more strongly interact with the water molecules than that of chloroethanol, since the F atom has the highest electronegativity. Because the hydrophobic hydration in the vicinity of the OH group will be more disturbed, the ${\tau_c}^h/{\tau_c}^0$ value of FEtOH becomes smaller. However, it is not thought that the effect of the OH group of FEtOH is particularly strong, because the ${\tau_c}^h/{\tau_c}^0$ value is larger than that of ethanediol (see Table 1). Therefore, we assume that the OH group of the halogenoethanols on the water structure is approximately the same as that of ethanol.

From the above discussion, relation (3) is explained based on the disturbing effect of the halogen atom on hydrophobic hydration.

Mizuno et al.[8,9], reported that the effect of the kind of halogen atom on the high-field chemical shift is in the order ClEtOH < BrEtOH < IEtOH. According to them, the breaking effect on the water structure becomes large in this order, and their result is opposite to the relation of Eq. 3. It appears that their meaning about structure breaking is different from the accepted definition[22–24] on water structure breaking and making.

Hertz's group[25,26] found a high-filed chemical shift of water protons in aqueous tetraalkyl-ammonium salt solutions, and concluded that the hydrophobic structure forming effect is connected to hydrogen bond breakage. Consequently, the halogenoalcohols are hydrophobic structure makers.

Dynamics of Hydration of Polyhalogenoalcohols and Size Effect. As can be seen in Table 1, the ${\tau_c}^h/{\tau_c}^0$ values for halogenoalcohols increase with increasing the number of halogen atoms, although all of the values are smaller than those of the corresponding alcohols:

$$\mathrm{EtOH} \geq \mathrm{F_3EtOH} > \mathrm{FEtOH}, \quad (5)$$

$$\mathrm{EtOH} \geq \mathrm{Cl_3EtOH} > \mathrm{Cl_2EtOH} \geq \mathrm{ClEtOH}, \quad (6)$$

$$(CH_3)_2CHOH > (CF_3)_2CHOH. \quad (7)$$

As shown in Fig. 4, the hydrophobic hydration is disturbed by the adjacent halogen atom. As the number of halogen atom in a solute molecule increases, the interaction between water molecules and the halogen atom predominates. Since the rotational motion of the water molecules is more inhibited, the τ_c^h/τ_c^0 value increases with increasing the number of halogen atoms.

The τ_c^h/τ_c^0 values for halogenoalcohols are plotted as a function of ASA in Fig. 5. For a comparison, the same relations for alcohols and diols[14] are shown in Fig. 5.

The τ_c^h/τ_c^0 values for fluoro- and chloro-ethanols are expressed by

$$\tau_c^h/\tau_c^0 = -5.33 + 0.032\ (\mathrm{ASA}) \quad (8)$$

for fluoroethanols (FEtOH, and F_3EtOH) and

$$\tau_c^h/\tau_c^0 = 0.66 + 0.0036\ (\mathrm{ASA}) \quad (9)$$

for chloroethanols (ClEtOH, Cl_2EtOH, and Cl_3EtOH). The ASA dependence of τ_c^h/τ_c^0 for chloroethanol is smaller than that for fluoroethanols. The effect of the size of the halogen atoms also appears in the case of monohalogenoethanols. That is, the τ_c^h/τ_c^0 values increase a little from chloroethanol to iodoethanol, as shown in Fig. 5.

We found that the τ_c^h/τ_c^0 values for the homologues, such as oligosaccharrides,[27] amines, diamines, alcohols, and diols,[14] increase with increasing ASA, and then show the maximum, or approach an approximately constant value. Such a relation between the hydration and the size of the solute is due to the size effect. In the dissolution process of the solute molecule into water, the rearrangement of the water molecules occurs around the solute molecules. If the molecular size of the solute is small, the hydrogen bonds between those water molecules which surround the hydrophobic group are enhanced. In the case of a large solute particle, however, the rearrangement of water molecules is insufficient to maintain the same degree of hydrogen bonding, i.e. hydrogen bonds between the water molecules are destroyed.[27] Kjellender and Marcelja[28] showed that close to a large hydrophobic surface the number of hydrogen bonds between water molecules decreases. Lazaridis and Paulaitis[29] found that the orientational entropy per water molecule in the hydration layer of the spherical hydrophobic particle shows a maximum at an effective diameter of 4 Å. In other words, the rotational correlation time of water molecules in the hydration layer is maximum at this diameter. Generally speaking, in the case of homologues, the reorientational motion is inhibited with increasing ASA, and then shows a maximum, or approaches a value that is approximately constant (size effect).[13,14,27]

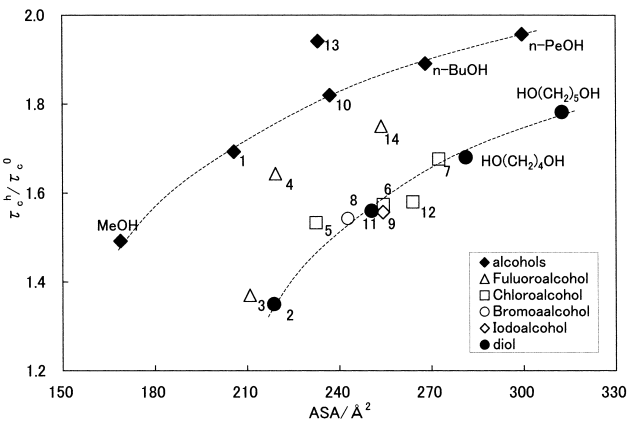

Fig. 5. Relationship between τ_c^h/τ_c^0 and ASA of the halogenoalcohols, alcohols and diols. The numbers demote the same compounds in Table 1.
◆ : Alcohols, △ : Fuluoroalcohols, ○ : Chloroalcohols, □ : Bromoalcohols, ◇ : Iodoalcohols, ● : Diols.

Interaction between Protein and Halogenoethanols. Although halogenoalcohols affect protein conformations, there have not been many investigations, except for F_3EtOH, and $(CF_3)_2$CHOH.

It is necessary to see the effects of other halogenoalcohols on the stabilization of proteins in order to elucidate the relation between the alcohol-induced denaturation of proteins and the hydration of halogenoalcohls. It is useful to compare the effectiveness of halogenoalcohols with their dynamic hydration number, by which the hydration characteristics of the solute are systematically represented.[13–15,17,27]

The inhibition of the activity of α-Chimotrypsin by halogenoethanols decreases in the order[30]

$$\mathrm{BrEtOH} > \mathrm{ClEtOH} > \mathrm{FEtOH} \quad (10)$$

and

$$\text{tri-} > \text{di-} > \text{mono-halogenoethanols.} \quad (11)$$

The degree of conversion of the β-sheet protein concanavalin A into an α-helix by halogenoethanols decreases in the order[31]

$$Cl_2\mathrm{EtOH} > \mathrm{BrEtOH} > F_3\mathrm{EtOH} > \mathrm{ClEtOH} > \mathrm{FEtOH}. \quad (12)$$

Jackson and Mantsh[31] suggested that this order is related to the combination of a relatively low dielectric constant and a high dipole moment.

Relations (11)–(13) are consistent with the order of n_{DHN} (see Table 1).

Hirota et al.[32] studied the alcohol-induced conformational transition of melittin by various alcohols and halogenoalcohols. For example, the effect of monohalogenoalcohlls and ethanol decreases in the order

$$\mathrm{BrEtOH} > \mathrm{ClEtOH} > \mathrm{EtOH} > \mathrm{FEtOH}. \quad (13)$$

The values of the alcohol concentration (C_M), which corresponds to the midpoint of the sigmoid curve of the ellipticity at 222 nm for these alcohols, are ca. 1, 2.5, 5 and 6 M, respectively. The values of C_M for only halogenoalcohls are expressed by the linear equations of n_{DHN} or ASA:

$$C_M = 12.8\text{–}1.21\ n_{DHN}\ (r = 0.99), \quad (14)$$

$$C_M = 39.1–0.157\,\mathrm{ASA}\ (r = 1.0). \quad (15)$$

In addition, the effects of fluoroalcohols and alcohols on the conformation change of fovine β-lactoglobaline and melittin decrease in the order[2]

$$(CF_3)_2CHOH > F_3EtOH > i\text{-PrOH} > EtOH > MeOH. \quad (16)$$

Relation 17 is subdivided into 2 groups of fluoroalcohols and alcohols based on n_{DHN} (or ASA). The above results suggest that although the hydration of the solute is an important factor which governs the solute-induced denaturation of proteins, the mechanism of the interaction by halogenoalcohols is different from that by alcohols.

Conclusion

The τ_c^h/τ_c^0 value for halogenoalcohols confirms that these molecules are the hydrophobic structure maker and that the degree of the dynamic hydration depends on the kind and number of halogen atoms. The τ_c^h/τ_c^0 values for monohalogenoethanols decreases in the order IEtOH $\geq$ BrEtOH $\geq$ ClEtOH $>$ FEtOH; this order corresponds to that of the electronegativity of the halogen atom. Although values for polyhalogenoalcohols increase with increasing the number of halogen atom, their values are smaller than those for the corresponding alcohols. The results are due to the disturbing effect of the halogen atoms on hydrophobic hydration and their size effect.

References

1 S. Lapanje, "Physicochemical Aspect of Protein Denaturation", Wiley-interscience, New York (1978).

2 N, Hirata, K. Mizuno, and Y. Goto, *Protein Sci*., **6**, 416, (1997).

3 J-S. Chiou, T. Tatara, S. Sawamura, Y. Kaminoh, H. Kamaya, A. Shibata, and I. Ueda, *Biochim. Bioplays. A.cta*, **119**, 211, (1992).

4 M. Buck, *Q. Rev, Biophys*., **31**, 297, (1998).

5 W-Y. Wen and J. A. Muccitelli, *J. Solution Chem*., **8**, 225, (1979).

6 P. M. Claesson and H. K. Christenson, *J. Phys. Chem*., **92**, 1650, (1985).

7 N. Muller, *J. Pharm. Sci*., **75**, 987, (1986).

8 K. Mizuno, K. Oda, S. Maeda, and A. Okamura, *J. Phys. Chem*., **99**, 3056, (1995).

9 K. Mizuno, K. Oda, Y. Shindo, and A. Okamura, *J. Phys. Chem*., **100**, 10310, (1996).

10 C. H. Rochester, and J. R. Symonds, *J. Chem. Soc., Faraday Trans*., **I69**, 1274, (1973).

11 M. Fioroni, K. Burger, A. E. Mark, and D. Roccatano, *J. Phys. Chem*., **B104**, 12347, (2000)

12 R. Chitra, and P. E. Smith, *J. Chem. Phys*., **114** 426, (2001).

13 S. Okouchi, T. Moto, Y. Ishihara, H. Numajiri, and Hi. Uedaira, *J. Chem., Soc*., *Faraday Trans*., **92**, 1853, (1996).

14 Y. Ishihara, S. Okouchi, and Hi. Uedaira, *J. Chem., Soc*., *Faraday Trans*., **93**, 3337, (1997).

15 Hi. Uedaira, M. Ikura, and Ha, Uedaira, *Bull. Chem. Soc. Jpn*., **62**, 1, (1989).

16 A. Bango, G. Lovato, G. Seorrano, and J. Wijinen, *J. Phys. Chem*., **97**, 4601, (1993).

17 Hi. Uedaira and Ha. Uedaira, *Curr. Top. Solution Chem*., **1**, 39, (1994).

18 W. Blokzijl and J. B. F. N. Emgberts, *Angew. Chem., Imt. Edn. Engl*., **32**, 1545, (1993).

19 Hi. Uedaira and Ha. Uedaira, *Zh. Fiz. Khim*., **42**, 3024, (1968).

20 M. Ishimura and Hi. Uedaira, *Bull. Chem. Soc. Jpn*., **63**, 1, (1990).

21 Hi. Uedaira and Ha. Uedaira, *Nippon Kagaku Kaishi*, **1986**, 1265.

22 R. W. Gurney, "Ionic Prosesses in Solution", McGraw-Hill, New York, (1953).

23 O. Ya. Samoilw, *Discuss. Faraday. Soc*., **24**, 141, (1957).

24 H. G. Hertz, in "Water: A comprehensive Treatise", ed by F. Franks, Plenum Press, New York (1973), Vol.3, Chap. 7.

25 H. G. Hertz and W. Spalthoff, *Z. Elektrochem. Ber. Bunsenges. Phys. Chem*., **68**, 1096, (1964).

26 J. Diratsaoglu, H. Hauber, H. G. Hertz, and K. J. Müller, *Z. Physik. Chem. Neue Folge*, **168**, 13, (1990).

27 Hi. Uedaira, M. Ishimura, S. Tsuda, and Ha. Uedaira, *Bull. Chem. Soc. Jpn*., **63**, 3376, (1990).

28 R. Kjellender, and S. Marcelja, *Chem, Phys. Lett*., **120**, 393, (1985).

29 T. Lazaridis, and M. E. Paulaitis, *J. Phys. Chem*., **98**, 635, (1994).

30 S. Ebina, T. Kobayashi, and Y. Nagai, *Experimenta*, **35**, 1011, (1979).

31 M. Jackson and H. H. Mantsch, *Biochim. Biophys. Acta*, **1118**, 139, (1992).

32 N. Hirota, K. Mizuno, and T. Goto, *J. Mol. Biol*., **275**, 365, (1998).